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ASSIMILATION OF RADIOACTIVE PHOSPHORUS BY *LIRIOMYZA TRIFOLII* (BURGESS) (DIPTERA: AGROMYZIDAE) FROM FEEDING AT DIFFERENT TEMPERATURES ON DIFFERENT CHRYSANTHEMUM CULTIVARS

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Abstract.—Measurements of whole-body retention of ^{32}P by *Liriomyza trifolii* (Burgess) from feeding on tagged *Chrysanthemum morifolium* Ramat cultivars indicated that assimilation of ^{32}P was greatest from feeding on 'Capri' and 'Sunny Mandalay' and less from 'Spice', 'Mandarin', 'Dramatic', 'Garland', and 'Minute Man'. Females assimilated at least 13% more ^{32}P than males at 20, 25, 30, and 35°C. Metabolic rates doubled with each 10 degree increase in temperature; larval developmental rates varied with temperature while gross assimilation and amount of leaf tissue consumed were identical at all temperatures.

Agromyzid leafminers are found throughout the world, with 206 described North American species, represented in 15 genera and 11 subgenera (Spencer, 1981). The genus *Liriomyza*, with 233 described species, is the third largest in the world (Spencer, 1981). Several species of *Liriomyza* are of particular economic importance in the United States and elsewhere due to their destructive nature and status as economic pests. *Liriomyza trifolii* (Burgess) has a wide host range feeding primarily on the Compositae (23 host genera), followed by Fabaceae (6 genera) (Powell, 1981). Over 47 genera in 10 families are utilized as a food source in Florida alone (Spencer, 1981).

Liriomyza trifolii first-instar larvae hatch after several days and immediately begin to mine. The leaf mine varies depending on the host plant but is generally long, linear, narrow and not greatly widening at the ends, with strips of frass on alternate sides of the tunnel or in granular patches or connected threads (Spencer, 1973). Feeding on the leaves of the upper part of the chrysanthemum plant reduces salability of the crop. Mines may also reduce photosynthesis (Parrella et al., 1985) or provide infection sites for pathogens (Schuster and Harbaugh, 1979). Two molts and three larval instars take place within the leaf tissue. The third-instar larva emerges from the leaf after about a week, generally drops to the ground, and molts a final time within the puparium. The adult emerges from the puparium in 9 to 18 days, depending upon the temperature.

The female fly lays an egg by first selecting a site on a suitable leaf, elevates her head and lowers the abdomen. The body is held almost perpendicular to the leaf and by rapid piercing movements of the abdomen, the epidermis is punctured.

A rotary movement of the abdomen enlarges the opening to 0.35 mm in diameter, and an egg is laid parallel to the leaf surface in the cellular tissue. Regardless of oviposition or not, the female backs up and feeds on the cellular fluid that exudes from the stipple. Males also feed at these wounds or on exudations from leaf axils.

Liriomyza trifolii exhibits an intercultivar preference when feeding on Chrysanthemum (*Chrysanthemum morifolium* Ramat) (Oetting, 1982). A determination of host plant suitability is needed, so that the most resistant cultivars can be identified for use in integrated pest management schemes or used in breeding programs. This experiment was performed to determine assimilation at varying temperatures and then, using an optimum developmental temperature, determine assimilation of phosphorus in different chrysanthemum cultivars. Assimilation by larvae of an essential and readily absorbed plant and insect element, phosphorus, was used to determine host utilization. Assimilation, in this study, is defined as the whole-body retention of phosphorus-32 (^{32}P). Insect health determinations were then used to rank chrysanthemum cultivars by feeding assimilation to attain the objective of determining the suitability of hosts.

Materials and Methods

Assimilation studies were performed during the spring of 1982 in Athens, Georgia. A total of 50 cuttings, 10 cuttings per temperature treatment, of *Chrysanthemum morifolium* Ramat, cultivar 'Mandarin', were used for measurements of ^{32}P assimilation at 20, 25, 30, 35, and 40° C ± 2° C. Plastic 29 ml rearing cups with cardboard tops were used to maintain plants throughout the study. A 6 mm diameter hole was punched through each lid and the bare-rooted cutting inserted through this into the cup. Two millicuries of ^{32}P, as phosphoric acid, were added to 250 ml of water. Twenty-five ml were placed in each cup and the roots were suspended in it. Plants were allowed to absorb this solution for 1–3 days in order to assimilate as much ^{32}P as possible. Plants were then transferred to and maintained in uncontaminated, water-filled cups so that absorption of tagged water ceased and radioactive decay could be followed. Groups of 5 plants were placed in 3.8 liter cylindrical cardboard tubs and the tops were sealed with clear 12 mil polyethylene. Flies were collected with an aspirator from a stock colony maintained in a greenhouse of chrysanthemums. These flies were immediately immobilized by chilling in a refrigerator. Groups of 10 females and 1 male were sorted and placed into 20 dram vials. One vial of flies per tub was then screwed into a port in the side of the tubs to introduce insects to plants. After 24 hours, flies were anesthetized with CO_2 and killed. A 6.35 mm diameter leaf disc was removed from the bottom-most leaf of each plant with a paper punch to act as a standard for measurements of radioactivity. The plants in their water filled cups were individually placed on 14.7 cm diameter petri dishes and transferred to their respective environmental chambers. One Percival, two Environator and one Precision environmental chamber were used throughout this experiment. A 14:10 L:D hr photoperiod was maintained in each.

Leaf discs were assayed for radioactive decay over time by 1 minute counts of Bremsstrahlung on a Tracor Northern TN-1705 multichannel pulse height analyzer (Willis et al., 1975). Samples were placed in a well-type, solid scintillation detector with a NaI(Tl) crystal. Counts were summed over channels 10–40 at 1091 volts 7nd gain of 4.0. When all surviving last-instar larvae emerged from

the leaves, they were placed seperately into test tubes, placed into the detector well, and assayed for radioactivity. Background counts of 5 minutes were converted to counts per minute and subtracted from leaf and larval counts to correct for natural emissions. Disintegrations per minute (dpm) were determined by dividing by the counting efficiency (2.44%). Each dried leaf and larva or pupa was weighed to the nearest 0.0001 mg on a Cahn/Ventron Cahn 21 automatic electrobalance. This value was used to standardize data as dpm/wt. The leaf disc data were transformed (ln) and least squares regression performed to approximate a slope of −14.3 (days), the half-life of ^{32}P. Percent of ^{32}P in the leafminer was determined by identifying the point on the decay line that corresponded to the point for the leafminer at the time the leafminer was assayed. The dpm/wt of the line point was subtracted from the dpm/wt of the larva and divided by the larva dpm/wt, then multiplied by 100 to give a value for percent retention.

Leaf area consumed was digitized in mm^2 by a Hewlett Packard 9825A computer with a 9864A Digitizer attachment, for correlation between area consumed, weight consumed and retention of ^{32}P. Area consumed per larva was determined by tracing the leaf portion skeletonized by each larva on a light table and digitizing it. Weight consumed was determined by correlating the mean area of 20 6.35 mm diameter leaf discs (30.6 ± 1.2 mm^2) with the mean weight of leaf discs for each set of 5 replicates. This value was then multiplied by the area consumed per larva to provide individual consumption values. Five plants for each temperature of 20, 25, 30 and 35° C were counted from 17 April 1982 to 9 May 1982. The second replicate of 5 plants was counted from 3 May 1982 to 22 May 1982. Plants were monitored from 15 May to 27 May for assimilation studies at 40° C. Data were analyzed by analysis of variance and comparisons of means were made using the Student-Newman-Keuls test at the 0.05 level.

Six additional cultivars, 5 replicates each of 'Capri', 'Dramatic', 'Garland', 'Minute Man', 'Spice', and 'Sunny Mandalay' were used for inter-cultivar assimilation studies. Flies were allowed to feed and oviposit as above for 32 hours; counting then started on 8 June 1982 until 23 June 1982. Because of low level of infestation, a second exposure of flies (>15 females) was allowed on the same plant 24 June 1982 for 32 hours, followed by a third exposure for 32 hours on 1 July 1982 (>30 females). All cultivars were maintained at 30° C ± 2° C.

Percent gross ^{32}P assimilation per larva was determined as described above. The number of mines initiated and realized natality for each group of cultivars were determined by actual count. Data were analyzed by analysis of variance and comparisons of means were made using Duncan's New Multiple Range Test.

Results and Discussion

Percent gross assimilation of ^{32}P was not significantly different but shows a tendency to increasing with increase of temperature, but the mean leaf area consumed, the mean weight consumed and the mean weight per larva are not significantly different (Table 1). Only rate of assimilation would be expected to increase with increasing temperature because of biochemical, especially enzymatic, activity. At high temperatures, proteins denature and the efficiency of physiological processes is reduced. For this reason assimilation would be expected to decrease sharply. This occurred at 40° C in this experiment. Under this condition, eggs were observed to hatch and an increase in mine length noted for one

Table 1. Percent assimilation/larva of ^{32}P, mean area and weight consumed/larva, mean larval weight, days to larval drop, and sex ratio of *Liriomyza trifolii* (Burgess) reared on *Chrysanthemum morifolium* Ramat 'Mandarin' containing ^{32}P.

Parameter Measured	Temperature			
	20° C	25° C	30° C	35° C
Gross Assimilation (%)	89.42ab[1]	83.52b	90.71ab	93.98a
Female Assimilation (%)	93.79a	92.37a	94.48a	97.37a
Male Assimilation (%)	80.69a	68.76b	80.02a	78.75ab
$\bar{x}$ Leaf Area Consumed (mm^2)	164.03a	170.82a	184.08a	161.24a
$\bar{x}$ Weight Consumed (mg)	4.86a	5.36a	6.65a	5.08a
$\bar{x}$ Larval Weight (mg)	0.2369a	0.2055a	0.1683a	0.2086a
$\bar{x}$ Days to Larval Drop	13.67a	11.57a	7.60b	7.00b
Sex Ratio (Female : Male)[2]	2:1	1.6:1	2.8:1	4.5:1

[1] Means are compared horizontally; those followed by a letter in common are not significantly different ($P = 0.05$) using the Student-Newman-Keuls test.

[2] Number of adults to emerge in respective temperatures was 20° C (18), 25° C (16), 30° C (25), and 35° C (11).

day before death occurred. The physiological state of the host plant may also have had detrimental effects on leafminer development, as death of all leafminers occurred and death of all plants was accelerated when maintained at 40° C.

Temperature significantly affects developmental time from egg to pupation (Leibee, 1984). Time from egg hatch to pupation, in our tests, was almost two times greater at low temperatures than the higher temperatures. The Q_{10} law, that for every 10 degree increase in temperature there is a doubling of biochemical reaction rate (King, 1965), is complied with. Since each larval stadia has a fixed number of cells and growth occurs only by cell enlargement and cell elongation, assimilation rate increases while total assimilation at all temperatures is the same.

Possibly correlated with temperature are the sex ratios of females to males. As temperature increased, female survivorship was favored. This was possibly due to the detrimental effects high temperatures have on all the developmental stages. Under stressful environmental conditions, females, with the potential for reproductive success, need to survive in order to maintain the species. Males are not as necessary since even with lower numbers their polygamous nature insures that the majority of females will be mated.

A drop in gross assimilation occurred at 25° C. Female leafminers, in all treatments, assimilated the same amount of ^{32}P, and assimilated at least 13% more than their male counterparts. Extremely low assimilation by males at 25° C accounted for the low gross assimilation value, otherwise gross assimilation and assimilation by males was the same in all treatments.

Females assimilate more ^{32}P, due not only to a greater body weight, but also to an intrinsically higher uptake (Srinivasan et al., 1980), which was also observed by Chamberlain (1979) in *Stomoxys calcitrans*, Quraishi et al. (1966) in *Anopheles stephensi mysorensis*, and by Quraishi (1968) in *Aedes aegypti*. Controversy over utilization of phosphorus exists. Whittaker (1961) generalizes that much of the ^{32}P that is ingested by an organism is not assimilated while Suzuki (1978) found that conversion efficiency by *Locusta migratoria* was 52%. Phosphorus is one of the chief constituents of insect protoplasm and phosphorylated imtermediates are

Table 2. Percent gross assimilation of ^{32}P, initial natality, and realized natality of *Liriomyza trifolii* (Burgess) reared at 30° C on 7 cultivars of *Chrysanthemum morifolium* Ramat.

	Cultivar						
	Sunny Mandalay	Capri	Spice	Mandarin	Dramatic	Garland	Minute Man
Gross Assimilation (%)	98.65a[1]	94.15	92.14	90.74b	90.42b	87.98b	84.43
Initial Natality[2]	33.80bc	27.20d	57.25a	36.20b	37.50b	13.67d	29.40cd
Realized Natality[3]	3	1	1	25	9	6	1

[1] Means are compared horizontally; those followed by a letter in common are not significantly different ($P = 0.05$) using Duncan's New Multiple Range Test. Those values with n = 1 cannot be tested by an analysis of variance.

[2] Initial natality based on the number of mines initiated.

[3] Realized natality based on the number of larvae to emerge from each plant.

intimately involved in nerve functions. A significant proportion of ^{32}P accumulates in the thorax and abdomen (Srinivasan et al., 1980). This need for phosphorus is basic to both sexes, but Srinivasan et al. (1980) postulate that the greater female need is due to ovarian development. This was demonstrated by Smittle and Patton (1970) when they found that female *Culex pipiens quinquefasciatus* retained only 60% of their radioactivity after ovipositing.

'Capri' and 'Sunny Mandalay' were probably the preferred chrysanthemum cultivar hosts of *L. trifolii* based on gross assimilation of ^{32}P (Table 2).

Low numbers of leafminers were reared on several of the cultivars and the assimilation value for these treatments may be inflated or deflated depending on whether the individual reared was a female or male respectively. The poor success in rearing larvae to pupation may have been due to the physiology of the host or to ^{32}P induced death. Death at hatching would account for the erratic values of the number of mines initiated for each treatment. Many workers report retarded growth or a reduced hatch rate if concentrations of ^{32}P in rearing media are high (Abdel-Malek, 1961; Dustan, 1966; Chamberlain, 1976). Lethality due to ^{32}P was unlikely, since the amount of ^{32}P utilized was small, and identical to the amount used in the preceding experiment.

The technique of determining host preference based on assimilation of ^{32}P can be utilized for a simple control program. If hosts that are poor in providing nutrients to developing leafminers are utilized in breeding programs for new chrysanthemum cultivars, this quality may be imparted to the new cultivar and aid in leafminer suppression. This technique can also be used to identify preferred hosts, of non-economic importance, to be used for trapping.

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